DEVELOPMENT OF HEALTH CRITERIA FOR SCHOOL SITE RISK ASSESSMENT PURSUANT TO HEALTH AND SAFETY CODE SECTION 901(g):

PROPOSED CHILD-SPECIFIC REFERENCE DOSES (chRDs) FOR SCHOOL SITE RISK ASSESSMENT – Cadmium, Chlordane, Heptachlor/Heptachlor Epoxide, Methoxychlor, and Nickel

DRAFT REPORT

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Integrated Risk Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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DRAFT FOR PUBLIC REVIEW
Proposed chRDs for School Site Risk Assessment

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Proposed chRDs for School Site Risk Assessment

Executive Summary

As mandated by Part 2 of the Health and Safety Code, section 901(g), the Office of Environmental Health Hazard Assessment (OEHHA) reviewed five chemicals to consider the development of child-specific reference doses (chRDs). This report summarizes OEHHA's review of pertinentscientific studies in proposing these chRDs . Any chRDs established as a result are intended for use in the risk assessment of proposed or existing California school sites.

OEHHA completed Part 1 of that mandate, which called for the identification of chemical contaminants commonly found at school sites and determined to be of greatest concern to children. The report, "Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code, Section 901(g): Identification of Potential Chemical Contaminants of Concern at California School Sites," was posted on OEHHA's website in June, 2002. In summary, OEHHA identified seventy-eight chemicals that will likely be found as contaminants of California school sites and have the potential for causing adverse effects in children. This should be viewed as a living compilation – chemicals may be added or removed as new information becomes available.

OEHHA chose five chemicals from the compilation for an in-depth evaluation of non-carcinogenic effects:cadmium, chlordane, heptachlor and its metabolite heptachlor epoxide, methoxychlor, and nickel. The criteria used to select these chemicals for the first round of reviews are discussed in Chapter 1.

In reviewing the applicable scientific literature, OEHHA identified relevant quantitative studies from which to propose a chRD for each chemical. The chRD for the non-carcinogenic effects of cadmium is based on a 1999 study by Buchet et al. The authors reported a strong relationship between cadmium body burden and renal tubular dysfunction in adult humans. This study identified a lowest observed adverse effect level (LOAEL) of 1 x 10^{-3} mg/kg-day. From this LOAEL, OEHHA calculated a chRD of 1 x 10^{-5} mg/kg-day using an uncertainty factor of 30 (10 to account for human variability and 3 to extrapolate from the LOAEL to the no observed adverse effect level (NOAELA factor of 3 (rather than the usual default of 10) was used for extrapolating from a LOAEL to a NOAEL because the LOAEL was based on the minimal adverse effect observed. A child protective modifying factor of 3 was used to account for the child/adult difference in gastrointestinal absorption of cadmium for a combined uncertainty and modifying factors of 90.

The chRD for the non-carcinogenic effects of chlordane is based on a 1994 study by Cassidy et al. The authors demonstrated changes in sex-steroid mediated behaviors, including increased male-typical spatial abilities in female rats and increased male-typical mating behaviors in male rats, following pre- and postnatal exposure. This study identified a LOAEL of 0.1 mg/kg-day, from which OEHHA calculated a chRD of 3.33 x 10^{-5} mg/kg-day using an uncertainty factor of 3000 (10 for interspecies variability, 10 for human variability, 10 to extrapolate to the LOAEL from the NOAEL, and a modifying factor of 3 to account for an inadequate hematotoxicity/immunotoxicity and neurotoxicity database—toxicities to which children may be particularly sensitive).

The chRD for the non-carcinogenic effects of heptachlor is based on two studies. One is a 2001 study by Moser et al., which shows decreased performance on measures of cognitive function in male rats following pre- and postnatal exposure, through postnatal day 21. The other is a 2001 study by Smialowicz et al., which shows suppression of the primary IgM and secondary IgG antibody responses following exposure during the last half of gestation through puberty. Both studies identified a LOAEL of 0.03 mg/kg-day. OEHHA calculated a chRD of 3 x10^{-5} mg/kg-day using an uncertainty factor of 1000 (10 each for interspecies variability, human variability, and extrapolation from LOAEL to NOAEL). The chRD for the non-carcinogenic effects of heptachlor epoxide utilizes the same study selected by U.S. EPA for its reference dose (RfD) and OEHHA for its Public Health Goal (PHG.) A LOAEL of 0.0125 mg/kg-day for liver-to-body weight ratio was reported when adolescent dogs were fed heptachlor epoxide for 60 weeks (Dow Chemical Co., 1958). Since exposure was to adolescent animals, OEHHA utilized the U.S. EPA RfD for its chRD of 1.3 x 10^{-5} mg/kg-day and utilized the same uncertainty factor of 1000 (10 each for interspecies variability, human variability, and extrapolation from LOAEL to NOAEL).

The chRD for the non-carcinogenic effects of methoxychlor is based on two studies as well. One is a 1995 study by vom Saal et al., which demonstrates increased urine marking in male mice, an index of territorial behavior, subsequent to prenatal exposure. The other is a 1999 study by Welshons et al., which shows an increase in adult prostate size following prenatal exposure. The LOAEL identified from these studies is 0.02 mg/kg-day. OEHHA calculated a chRD of 2 x10^{-5} mg/kg-day using an uncertainty factor of 1000 (10 each for interspecies variability, human variability, and extrapolation from LOAEL to NOAEL).

The chRD for the non-carcinogenic effects of nickel is based on the observed pup mortality in three reproductive studies – Smith et al., 1993, and Springborn Laboratories, 2000 a and b. In reviewing these three studies in totality, OEHHA concludes that the 1.1 mg nickel/kg-day (5 mg nickel sulfate hexahydrate/kg-day) dose constitutes the appropriate NOAEL. From this NOAEL, OEHHA calculated a chRD of 3.7 x 10^{-3} mg/kg-day, using an uncertainty factor of 300: 10 each for interspecies extrapolation and human variability) and a child protective modifying factor of 3 to account for the child/adult difference in gastrointestinal absorption of nickel.

Table ES 1 below compares the chRDs and U.S. EPA's RfD, which are based on studies in adult animals.

Table ES 1 OEHHA's chRD and U.S. EPA's RfD

	OEHHA's Proposed chRD (mg/kg-day)	U.S. EPA's RfD (mg/kg-day)
Cadmium	1×10^{-5}	5×10^{-4}
Chlordane	3.3×10^{-5}	5×10^{-4}
Heptachlor	3×10^{-5}	5×10^{-4}
Heptachlor epoxide	1.3×10^{-5}	1.3×10^{-5}
Methoxychlor	2×10^{-5}	5×10^{-3}
Nickel	3.7×10^{-3}	2×10^{-2}

These proposed chRDs were reviewed internally. OEHHA is currently releasing this draft report for external peer review and public comment. Any chRDs established by this process are intended for use in risk assessment of proposed or existing school sites in California.

1. Introduction

This report summarizes the first-year effort of the Office of Environmental Health Hazard Assessment (OEHHA) in developing health criteria for five selected chemicals for use in school-site risk assessment. The following provides the context for this effort.

1.1 Mandate

As part of a series of legislative provisions in California to protect children from exposure to environmental contaminants, OEHHA has been tasked under Health and Safety Code, Section 901(g), to fulfill the following directives:

1. On or before January 1, 2002, OEHHA, in consultation with the appropriate entities within the California Environmental Protection Agency (Cal/EPA), shall identify those chemical contaminants commonly found at school sites and determined by OEHHA to be of greatest concern based on criteria that identify child-specific exposure and child-specific physiological sensitivities.

2. On or before December 31, 2002, and annually thereafter, OEHHA shall publish and make available to the public and other state and local environmental and public health agencies and school districts, numerical health guidance values for five of those chemical contaminants identified until the contaminants identified have been exhausted.

1.2 Identification of Chemicals

The first part of the mandate, identifying those chemical contaminants commonly found at school sites and determined to be of greatest concern based on criteria that identify child-specific exposures and physiological sensitivities, was completed earlier. Available data did not permit us to definitively identify those chemicals that are commonly found at school sites and for which children have unique physiological sensitivities. However, we have identified a group of candidate chemicals that will likely be found as contaminants at school sites (200 chemicals), and another group where evaluation of review literature provided some indication the chemicals may have the potential for causing adverse effects on school-age children (198 chemicals). The methods used to implement the first part of the mandate are summarized below; a detailed description can be found in the OEHHA June 2002 report, "Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code, Section 901(g): Identification of Potential Chemical Contaminants of Concern at California School Sites," which can be downloaded at:
http://www.oehha.ca.gov/public_info/public/kids/pdf/ChildHealthreport60702.pdf.

In identifying contaminants likely to be found at California school sites, OEHHA considered chemicals that have been targeted by federal and state agencies as being pervasive in pertinent environmental media, in addition to contaminants that have been found at school sites or were on the list of analytes for monitoring studies at California school sites. Using these criteria, OEHHA considered the following as candidate contaminants that are likely to be found at school sites:

- Ninety-four soil contaminants that have been reported in school site Preliminary Endangerment Assessments (PEA) and reviewed by the Department of Toxic Substances Control (DTSC).
- Ninety Toxic Air Contaminants that were emitted to, or detected in, California's ambient air.
- Sixty potential classroom contaminants targeted for monitoring by the Department of Health Services (DHS) and the Air Resources Board (ARB), as part of their Portable Classroom Program.
- Forty-seven toxic chemicals targeted by U.S. EPA in its National Human Exposure Assessment Survey (NHEXAS).
- Twenty-six contaminants targeted by U.S. EPA in its Total Exposure Assessment Methodology (TEAM) studies.

Because of overlap among the chemicals reported or targeted by the above federal and state agencies, the final compilation consists of 200 chemical contaminants likely to be found at school sites.

In identifying chemicals with potential child-specific sensitivities, OEHHA considered the following:

- Chemicals with one or more citations in review articles from the scientific literature indicating the potential for adverse effects on the development of the nervous, respiratory, reproductive, endocrine, or immune system.
- Chemicals that initiated cancer following exposure during the perinatal period or childhood.
- Chemicals identified as Proposition 65 Developmental and Reproductive Toxicants by OEHHA, excluding chemicals not likely to be found at school sites, such as pharmaceuticals. While Prop 65 deals with chemical exposures that occur during pregnancy, the inclusion of these Prop 65 chemicals are useful for screening chemicals that may also impact developing organ systems postnatally.

The final compilation consists of 198 chemicals to which children are potentially more sensitive, 87 of which are from the Proposition 65 list.

To aid in implementing the second part of the mandate, the two compilations were merged into one comprehensive list of 78 chemicals that met both criteria. This list is given in table ES 1 in OEHHA's June 2002 report (page 5) and will be updated as new data become available. The information obtained to produce this compilation is not

sufficient to conclude that the compiled chemicals are found in most schools or that children have a greater sensitivity compared to adults. The compilation has been prepared to assist OEHHA scientists in selecting chemicals for further in-depth review to determine if sufficient studies on toxicity to developing organ systems in the young exist to create a health guidance value specific for children. The list has no regulatory status.

1.3 Numerical Health Guidance Values

The second part of the mandate, which is the subject of this report, requires OEHHA to annually publish numerical health guidance values for five of those chemical contaminants identified in the June 2002 report until the contaminants identified have been exhausted. In order to prioritize chemicals from the compilations generated in Phase I for in-depth review, OEHHA outlined the following criteria, which was also described in the June 2002 report, for selecting chemicals for in-depth reviews. It should be emphasized that these four criteria are not permanent, but are being used to help us prioritize which chemicals to evaluate first. The compilation of chemicals will continue to be updated and reviewed.

1. Chemicals having a strong indication of their presence at school sites according to monitoring studies or other reliable sources.

2. Chemicals cited to have possible adverse effects in three or more of the systems that are undergoing critical development during childhood: the neurological, immunological, respiratory, reproductive, or endocrine systems.

3. Where applicable, chemical carcinogens with existing reference toxicity levels based on studies in adult animals that approximate the dose associated with a 10^{-4} (one in ten thousand) to 10^{-6} (one in a million) lifetime cancer risk.

4. Chemicals that other OEHHA programs have identified as a public health concern based on studies pertinent to children.

The first criterion addresses the possibility that children will be exposed to a given chemical while at school, since a chemical must have some probability of being found at a school site in order to warrant consideration under this mandate. The compilation of chemicals potentially found at school sites (OEHHA, 2002) was used to gauge the likelihood of a chemical being present at California school sites.

The second criterion helps select those chemicals that appear to have multiple effects on organ systems that are still undergoing development and maturation after birth. The nervous, immune, respiratory, reproductive and endocrine systems are being targeted because chemical insults at relatively low doses to any of these organ systems could

produce adverse effects, many of which may not be recognized until maturity (OEHHA, 2002).

In focusing on these critical organ effects, we have targeted non-cancer endpoints. In a separate task, OEHHA is developing a cancer evaluation methodology for children pursuant to HSC Section 901(e). Because that methodology will not be available until 2004, we are focusing on identifying and evaluating the non-cancer effects of chemicals. We will assess the cancer endpoint when the children's cancer methodology is developed.

The criteria for selecting chemicals identified in review articles as having effects on multiple developing organ systems was intended to increase the probability of identifying relevant literature during the in-depth search and review phase. Targeting chemicals with evidence of effects in three or more systems may miss an important developmental toxicant for which either only one system is affected or only one system has been adequately researched. However, given time and budget constraints, we felt this was an effective way to prioritize the chemicals on the list this year.

The third criterion helps target those chemicals that could conceivably be non-cancer risk drivers when new child-specific data were considered. If current data suggest that both carcinogenic and non-carcinogenic effects of concern occur at similar dose levels in adults, and studies on developing organ systems show that exposure may produce irreversible non-carcinogenic effects in children at dosages lower than those that are toxic to adults, then the chemical should have a high priority for further evaluation.

The fourth criterion allows us to build on related work done by other OEHHA programs. This year we utilized the peer-reviewed PHGs for drinking water contaminants developed by OEHHA. More information on the PHG process can be found at the web address http://www.oehha.ca.gov/water/phg/allphgs.html. The PHG effort provides an excellent springboard from which to conduct our current review for developing child-specific chRDs because the Pesticide and Environmental Toxicology Section's (PETS') reviews are recent and they cover the chemicals of interest.

Using the above criteria OEHHA selected cadmium, chlordane, heptachlor and its metabolite heptachlor epoxide, methoxychlor, and nickel as the first five chemicals for in depth review. Under contract with OEHHA, the Public Health Library at the University of California at Berkeley searched the following databases: PubMed, Toxline/DART, Excerpta Medicus (EMBASE), Chemical Abstracts, BIOSIS, International Pharmaceutical Abstracts (IPA), and ISI Web of Science. We searched by keywords that describe specific effects on developing organ systems. The keywords used in searching these databases are listed in Appendix A.

OEHHA evaluated the citations returned by the literature search (105 citations for cadmium, 65 for chlordane and heptachlor/heptachlor epoxide combined, 82 for methoxychlor, and 18 for nickel), and reviewed relevant qualitative background papers and quantitative studies. In addition, OEHHA reviewed pertinent studies cited in papers

obtained via the literature search. Because so few citations were returned for nickel, an alternate, very inclusive approach (discussed in Section 2.5) was used to ensure that we were not missing important studies.

1.4 Child-Specific Reference Dose (chRD)

U.S. EPA and the March of Dimes sponsored a workshop -- Identifying Critical Windows of Exposure for Children's Health -- in September 1999 to systematically review the state of knowledge on prenatal and postnatal exposures and subsequent outcomes (Environmental Health Perspectives Volume 108, Supplement 3, June 2000). In reviewing data on organ systems that are still undergoing development and maturation in children, workshop participants noted that data pertaining to children's sensitivities to environmental contaminants during various critical developmental periods are limited. In particular, very little attention has been given to peripubertal/adolescent exposures or adult consequences from childhood exposure. However, these limited data do suggest that children could be more sensitive than adults when their developing organ systems were exposed to harmful chemicals. For example, several developmental immunotoxicants (chlordane, dioxin, lead, and benzo[a]pyrene) demonstrate that perinatal exposures can produce toxicity at doses that do not affect adults and/or they produce irreversible changes which do not occur with adult exposure.

Endocrine disruptors, a group of chemicals that may produce differential toxicity to the young, have been the subject of much recent scientific and regulatory debate (Cranmer et al., 1984; Colborn et al., 1993; U.S. EPA, 1998). While not all chemicals reviewed in this first year are endocrine disruptors, the endocrine disruptors do pose a great concern because they could interfere with the proper hormonal signaling that is essential for growth and development of school children. An endocrine disruptor may be defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body (U.S. EPA, 1997). Exposure to endocrine disruptors during critical "programming" periods in development, in contrast to exposure during adulthood, may produce irreversible effects on the reproductive, nervous, and/or immune system (Bigsby, 1999). In adulthood, these endocrine disruptors might only produce reversible effects by participating in the "seesaw" process of stimulation and feedback inhibition.

The effects of endocrine disruptors are more difficult to evaluate than chemicals which have a direct toxic effect on an organ system. Endocrine disruptors can act directly on hormone receptors of developing target organs, or they can act indirectly by interfering with the synthesis or metabolism of natural hormones. An endocrine disruptor having a direct interaction with either intracellular receptors or membrane-bound receptors can mimic a natural ligand by acting as an agonist, or if it inhibits binding, as an antagonist. The best known examples are methoxychlor, chlordecone (Kepone), DDT, some PCBs, and alkylphenols (Mueller and Kim, 1978; White et al., 1994; U.S. EPA, 1997). The antiandrogenic action of vinclozolin is the result of the affinity of its metabolites for the

androgen receptor (van Ravenzwaay, 1992; Kelce et al., 1994). O,p-DDT and chlordecone inhibit binding to both estrogen and progesterone receptors (Laws, 2000).

An endocrine disruptor can have an indirect effect on a target organ if it changes the quantity of a hormone or the ratio of several hormones at target organs by altering hormonal storage and/or release, transport in the blood and clearance, or post-receptor activation. Many different mechanisms can produce these changes.

Disruption of normal signaling, whether it causes a direct effect on a target organ, or whether it alters hormone ratios or levels, can produce an adverse effect on a single organ system -- the reproductive, immune or nervous system -- if the disruption occurs during a critical period in development (Kavlock et al., 1996). Disruption of normal signaling can also produce adverse effects on multiple organ systems. For example, estrogen receptors can be found in the gonads, thymus, and brain; and the disruption of estrogen signaling could simultaneously affect the reproductive, immune, and nervous systems (Colborn et al., 1993; Diel, 2002). To complicate the matter further, one endocrine axis can impact another in a "cross-talk" (WHO, 2002). For example, in humans and some other mammals, most of the testosterone and estradiol circulating in the bloodstream are not bioavailable because of their binding to sex hormone binding globulins (SHBG). Not surprisingly, SHBG production is regulated by testosterone and estradiol (Prinsloo and Van Aswegen, 2000; Pugeat et al., 1996). However, via cross-talk, SHBG is also regulated by insulin. Thus, an insulin disruptor that causes an elevated insulin level would suppress SHBG production, leading to an increase in testosterone and estradiol bioavailability (Pugeat et al., 1996).

Given the complexity of hormonal signaling processes, it is also not surprising to find the relationship between dose and response to be another controversial issue. Endocrine disruptors often act by mimicking or antagonizing the actions of naturally occurring hormones that may be already at physiologically functional concentrations (WHO, 2002). The National Toxicology Program's Report of the Endocrine Disruptors Low Dose Peer Review concluded that biological changes occurred in the range of human exposures or at doses that are lower than those typically used in the EPA's standard testing paradigm for evaluating reproductive and developmental toxicity for endocrine active agents (http://ntp-server.niehs.nih.gov/htdocs/liason/LowDosePeerFinalrpt.pdf). Too little is known about the dose-response curves for immunotoxicity, neurotoxicity, or endocrine effects to decipher the independent or interactive effects of endocrine disruptors on these systems. The shape of the dose response curve varies with the endpoint and dosing regimen and it may be low-dose linear, threshold-appearing, or it may be shaped like an upright U or an inverted U (http://ntp-server.niehs.nih.gov/htdocs/liason/LowDosePeerFinalrpt.pdf; Markowski et al., 2001; vom Saal et al., 1997).

The above observations underscore some of OEHHA's challenges in implementing HSC Section 901(g) pertaining to the development of health criteria based on children's sensitivities. As discussed by Ginsberg et al. (2002) and Miller et al. (2002), the risks

that children incur from exposure to environmental contaminants could differ from adult risks as a result of the following factors:

- Greater exposure in young children from a higher inhalation and food ingestion rate per body weight; and greater contact with soil, house dust and other media that may contain contaminants.
- Pharmacokinetics differences between children and adults with respect to absorption, metabolism, clearance, protein binding, and volume of distribution, once exposure has occurred.
- Pharmacodynamic differences between children and adults; that is, the sensitivity of rapidly developing tissues/organs in children may differ from that in adults.

The exposure differences will be addressed under a separate task -- developing the school exposure assessment guidelines -- pursuant to HSC Section 901(f). Based on available pharmacokinetic and pharmacodynamic data, we will evaluate if existing U.S. EPA RfDs or OEHHA toxicity criteria are appropriate, or if child-specific ones should be proposed, for school-site risk assessment in California.

From a public health protection standpoint, OEHHA has adopted two policies in developing chRDs. First, in order to protect children from conception through the time they leave school, chRDs must consider school-aged children up to age 18; and infants and toddlers in daycare facilities located at school sites. Second, OEHHA opts to consider the most sensitive species and endpoints in our evaluations, meaning that the lowest LOAEL or NOAEL from available literature would be selected. Moreover, the paucity of data has underscored the reality that the databases for sensitive endpoints may be incomplete. An uncertainty factor for database deficiency will be considered as appropriate.

Our mandate, which is driven by environmental health policies, has charted OEHHA's course of action. We view this approach as an iterative process. Any chRD established will be subject to review and refinement as the state-of-the-science progresses.

2. Evaluation of Five Chemicals

This chapter is divided into five sections, one for each chemical reviewed in 2002. Each section provides information specific to the particular chemical, including background and exposure information; how the chemical meets our criteria for evaluation; existing pertinent health guidance values; findings from our literature review; and OEHHA's recommendation. The recommendation includes a discussion of the study (or studies) used in developing the chRD, the uncertainty and modifying factors used, the calculation, and the proposed chRD.

2.1 Cadmium

Cadmium is an important industrial chemical with diverse applications. It is used for the production of nickel-cadmium batteries, pigments, alloys, plastics, and synthetics. It is also used in metal plating. The Toxic Chemical Release Inventory of 2000 shows that 2,292 pounds of cadmium were emitted into the air, 792 pounds were discharged into surface water, 69,000 pounds were injected underground, and 663,895 pounds were released to land in the U.S. In comparison, 16 pounds of cadmium entered the air and 36,104 pounds were disposed of on land in California during 2000 (U.S. EPA, TRI2000).

Given its indestructible nature, cadmium persists in the environment, and can enter the food chain. OEHHA identified air, drinking water, soil, and food as the primary pathways for human exposure to cadmium (OEHHA, 1999a). U.S. EPA and ARB/DHS have deemed cadmium as a chemical of interest in their NHEXAS and Portable Classroom Study, respectively (OEHHA, 2002). ARB reported the occurrence of cadmium in California air and DTSC reported the presence of cadmium at 10 percent of the potential school sites reviewed by the Department, making it a relatively frequently observed contaminant.

In reviewing literature for the purpose of establishing a Public Health Goal (PHG) for cadmium in drinking water (OEHHA, 1999a), OEHHA found some evidence that cadmium may elevate blood pressure in both animals and humans. Renal toxicity of cadmium is well known. Cadmium tested positive in several mutagenic assays and in an epidemiological study; it was observed that individuals with higher levels of cadmium in their urine (>3 μg/L) had more frequent chromosomal aberrations in their lymphocytes. A number of studies in rats and mice indicated the developmental and reproductive toxicity of cadmium. Neurological and immune effects were also reported. Finally, tumors of the prostate, testes and hematopoietic system in the rat were associated with oral cadmium exposure; and human lung and prostate cancers had been associated with inhalation exposure. Thus, it is regarded as a potential human carcinogen by the oral route and a human carcinogen by the inhalation route.

OEHHA selected cadmium for an in-depth review in this first cycle not only from the standpoint of its exposure potential at school sites, but also because of its adverse effects

on various organ systems; some of which are still undergoing development in school children.

Pertinent Guidance Values

U.S. EPA RfD: 0.5 µg/kg-day (water) and 1.0 µg/kg-day (food)

U.S. EPA's RfD is based on cadmium's effect on the kidney. A concentration of 200 µg cadmium (Cd)/g wet human renal cortex is the highest renal level not associated with significant proteinuria (U.S. EPA, 1985). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) which results in 200 µg Cd/g wet human renal cortex; the model assumes that 0.01percent day of the Cd body burden is eliminated per day (U.S. EPA, 1985). Assuming 2.5 percent absorption of Cd from food or 5 percent from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 0.005 and 0.01 mg Cd/kg-day from water and food, respectively (i.e., levels which would result in 200 µg Cd/g wet weight human renal cortex). Thus, based on an estimated NOAEL of 0.005 mg Cd/kg-day for Cd in drinking water and an uncertainty factor (UF) of 10 that accounts for intra-human variability, an RfD of 0.0005 mg Cd/kg-day (water) was calculated; an equivalent RfD for Cd in food is 0.001 mg Cd/kg-day.

U.S. EPA gives a high confidence to its cadmium RfD. The choice of NOAEL does not reflect the information from any single study. Rather, it reflects the data obtained from many studies on the toxicity of cadmium in both humans and laboratory animals. These data also permit calculation of pharmacokinetic parameters of cadmium absorption, distribution, metabolism and elimination.

OEHHA PHG: 0.07 µg/L (a safe dose of 1 x 10^{-5} mg/kg-day)

OEHHA deemed the Buchet investigation (Buchet et al., 1990) as the best study for use in developing a Public Health Goal (PHG) for cadmium in drinking water. The study avoided the healthy worker effect by performing a cross-sectional examination on 1699 Belgian subjects between the ages of 20 and 80 years. The investigators found a strong relationship between cadmium body burden and renal tubular dysfunction. They observed a risk of renal effects at or above the urinary excretion rate of 2 µg cadmium/24 hours. Assuming an oral absorption rate of 5 percent and a daily excretion rate of 0.005 percent of body burden, Buchet estimated that this excretion rate corresponded to a mean renal cortex concentration of about 50 ppm or 50 µg/g (wet weight). In non-smokers (investigators' design to subtract a major source of cadmium from tobacco smoke), this level is reached after 50 years of an oral daily intake of 1.0 µg/kg body weight. As such, a LOAEL of 1.0 µg/kg-day was established.

OEHHA (1999a) applied this LOAEL in conjunction with an aggregated uncertainty factor (UF) of 100 (10 for intra-human variability, 3 for LOAEL to NOAEL extrapolation, and 3 for uncertainty in applying adult biokinetics to the entire age range from infancy to adulthood) for calculating a safe dose of 0.01 µg/kg-day. A factor of 3

(rather than the usual default of 10) was used for extrapolating from a LOAEL to a NOAEL because the LOAEL was based on the minimal adverse effect observed. The safe dose in turn was used to derive the PHG.

Current Evaluation Results

Because the cadmium data have recently been reviewed under the PHG process, we used the PHG review as a baseline for the current evaluation. Accordingly, we focused our literature search and review on the information that was not covered by the PHG evaluation. An attempt was also made to target literature pertaining to cadmium's effect on sensitive organ systems that are still undergoing postnatal development.

Based on the above search criteria, we compiled a list of references. From that list, we identified a number of papers relevant to cadmium's effects on testes and semen of rabbits exposed before and after puberty (Foote, 1999); pubertal and postpubertal cadmium exposure on the hypothalamic-pituitary-testicular axis function in rat (Lafuente et al., 2000); cadmium induction of apoptosis in the immune system (Tsangaris et al., 1998); postnatal cadmium exposure and long-term behavioral changes in rat (Smith et al., 1982); effects of cadmium and lead on cognitive functioning in children (Thatcher et al., 1982); and neurotoxic effects of cadmium in young rats (Wong et al., 1982). However, these were either qualitative/mechanistic, semi-quantitative, or quantitative studies with a LOAEL higher than that on which the PHG was based.

Recommendation

The renal effect of cadmium seems to be the most sensitive endpoint (with the lowest LOAEL), even when it is compared with developmental and reproductive study endpoints identified in the OEHHA PHG document or those identified by OEHHA's current evaluation. The rate of cadmium absorption affects its rate of accumulation in the kidney, and in turn its toxicity. Thus, available data suggest the PHG LOAEL should be retained for our current consideration. This LOAEL is lower than the NOAEL used by U.S. EPA in developing its RfD. Both the safe dose from which the PHG was derived and U.S. EPA's RfD are based on cadmium's effect on renal function. In developing these two health criteria, long-term cumulative exposure data were used. Both U.S. EPA and Buchet et al. applied a 5 percent absorption in their respective biokinetic modeling, based on adult human absorption of 4.7 to 7 percent of the cadmium intake (Rahola et al., 1972, cited in Mahaffey, 1983). In proposing the PHG, OEHHA applied a factor of 3 to account for the uncertainty associated with Buchet's modeling in which he applied adult biokinetics to the entire age range from infancy to adulthood. According to Alexander et al. (1974), the absorption of cadmium by children, from early infancy through 8 years of age, averages 55 percent.

To illustrate the appropriateness for applying the factor of three for developing the chRD, OEHHA ran a model similar to Buchet, using his modeling assumptions. Using a daily dose of 1 μg/kg, an absorption rate of 5 percent, and a daily clearance rate of 0.005

percent of body burden, OEHHA estimated that the urine excretion of 2 µg cadmium /24 hour (LOAEL biomarker) would be reached by age 53, which was in good agreement with Buchet's study. However, by changing the absorption rate to 55 percent through age 8, then decreasing it linearly to 5 percent at age 21, while keeping the other parameters the same, a daily dose of 0.51 µg/kg would be required to produce an urine excretion of 2 µg cadmium /24 hour by age 53. Thus, using child-specific absorption results in a difference of about 2-fold in the daily dose to produce the LOAEL effect. To be public health protective, OEHHA proposes to apply a child-protective factor of 3 to account for childhood absorption differences.

Calculation of the non-cancer chRD for cadmium is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF x CP}} = \frac{\text{1 µg/kg-day}}{\text{30 x 3}} = 0.01 \text{ µg/kg-day}$$

Where,

LOAEL = Lowest-observed-adverse-effect-level from Buchet et al., 1990.

UF = Uncertainty factor of 30 (10 for intra-human variability, 3 for LOAEL to NOAEL extrapolation because the LOAEL is based on the minimal effect observed and this is consistent with that applied to calculate the PHG).

CP = Child protective factor of 3 to account for the GI absorption difference between children and adults.

Accordingly, OEHHA is proposing a chRD of 0.01 µg/kg-day for cadmium's non-cancer effect to be used in school-site risk assessment instead of the U.S. EPA's RfD that did not account for a greater GI absorption of cadmium by children.

2.2 Chlordane

Chlordane is a cyclodiene pesticide, one of many organochlorine insecticides. Chlordane was used in large quantities until the U.S. EPA issued a notice of suspension except for use on subterranean structural termite control in 1976 (McConnachie and Zahalsky, 1992). It was banned for all uses in the United States in 1988, but it is still manufactured for export. Like DDT, it persists in the environment, and it is considered a priority persistent, bioaccumulative toxic (PBT) chemical by U.S. EPA (U.S. EPA, 2002).

Chlordane is not a pure chemical pesticide, and all studies investigating its toxicity or mechanism of action have used technical grade chlordane, which is a mixture. Infante et al. (1978) analyzed technical chlordane and reported that it contained 38-48 percent cis- and trans-chlordane, 3-13 percent heptachlor, 5-11 percent nonachlor, 17-25 percent other chlordane isomers, and a small amount of other compounds. Dearth and Hites (1991) identified 147 different compounds in a preparation of technical chlordane that included cis-chlordane (15 percent), trans-chlordane (15 percent), trans-nonachlor (15 percent), and heptachlor (3.8 percent).

OEHHA included chlordane in the "Compilation of Chemicals Potentially Found at School Sites" because it has been targeted by federal and state agencies as a chemical that may present environmental health risks. Chlordane appears on all but one of the chemical compilations that OEHHA has selected to identify chemicals that may be found at school sites. These compilations include:

- Soil contaminants identified at potential school sites in environmental investigations reviewed by the Department of Toxic Substances Control
- Toxic Air Contaminants (TACs) in California identified by OEHHA
- Analytes in the Department of Health Services/Air Resources Board (DHS/ARB) Portable Classroom monitoring study
- Analytes in the U.S. EPA National Health Exposure Assessment Study (NHEXAS)

Chlordane was placed by OEHHA in the compilation of "Candidate Chemicals Based on Critical Health Effects" because 1) it is on the Proposition 65 Developmental and Reproductive Toxin List and 2) a survey of recent scientific literature indicated that it possesses toxicity to organ systems that are developing in children, including the immune system, neuroendocrine and female reproductive systems (Ahmed, 2000; Barone et al., 2000; Barnett et al., 1990; Blyler et al., 1994; Brucker-Davis, 1998; DeRosa et al., 1998; Holladay et al., 2000; Holladay, 1999; Luster et al., 1990; Olea et al., 1998; Reigart, 1995; Spyker-Cranmer et al., 1982; Theus et al., 1992a and 1992b; Voccia et al., 1999). Chlordane exposure has also been associated with childhood cancer (Zahm et al., 1998.)

OEHHA staff prepared a PHG for chlordane in 1997 (OEHHA, 1997). The study on which the PHG is based showed that chlordane acted as an endocrine disruptor and

altered sex steroid-mediated behaviors when exposure occurred during gestation and lactation (Cassidy et al., 1994).

Endocrine disruptors, such as chlordane, are the subject of recent scientific and regulatory concern (U.S. EPA, 1998). They mimic or antagonize estrogens, androgens, and thyroid hormones, as well as their antagonistic analogs, and consequently disrupt the processes or tissues these hormones affect. Organ systems responsive to the sex steroids include the male and female reproductive organs, the central nervous system, and the immune system. The thyroid hormones affect most tissues (Bigsby, 1999). They are of particular concern in regard to children's health because they may disrupt the action of estrogen, androgen and thyroid hormones during critical periods of development and lead to permanent alterations in the reproductive, nervous, and immune systems that are developing during prenatal growth and childhood (Bigsby, 1999).

Existing Health Guidance Values

U.S. EPA Carcinogen Slope Factor: 3.5×10^{-1} per mg/kg-day

Chlordane is classified as B2; probable human carcinogen, using the 1986 Guidelines for Carcinogen Risk Assessment (Integrated Risk Information System (IRIS), 2003, http://www.epa.gov/iris/subst/0142.htm#carc). IRIS also reports that "under the 1996 Proposed Guidelines, it would be characterized as a likely carcinogen by all routes of exposure. These characterizations are based on the following summaries of the evidence available: (1) human epidemiology studies showing non-Hodgkin's lymphoma in farmers exposed to chlordane and case reports of aplastic anemia; chlordane associated with home use are inadequate to demonstrate carcinogenicity; (2) animal studies in which benign and malignant liver tumors were induced in both sexes of four strains of mice and occurred with an elevated, but not statistically significant, incidence in a fifth strain, as well as liver toxicity but no tumors in rats of two strains; and (3) structural similarity to other rodent liver carcinogens." The U.S. EPA oral slope factor is 3.5×10^{-1} per mg/kg-day. This value represents the geometric mean for five data sets with a range from individual data sets of 1.1×10^{-1} to 8.6×10^{-1} using the linearized multistage model (http://toxnet.nlm.nih.gov/). The EPA IRIS data base reported that the studies are of good quality and "the confidence is high that chlordane is a mouse liver carcinogen at dietary concentrations above 10 ppm. Although there is indication that the dose-response curve is sublinear in the dose region between 5 and 60 ppm, linearity at low doses cannot be ruled out on theoretical grounds. The tentative evidence is that the hematopoietic system, rather than the liver, is the target organ in humans."

U.S. EPA RfD: 5×10^{-4} mg/kg-day

The oral RfD established by U.S. EPA is 5×10^{-4} mg/kg-day based on a NOAEL of 0.15 mg/kg-day and LOAEL of 0.75 mg/kg-day in a mouse study (Khasawinah and Grutsch, 1989). The critical effect for the LOAEL was liver necrosis, with an uncertainty factor of 300 (10 for interspecies extrapolation, 10 for human variability, and 3 for deficiencies in

the database). The overall confidence given this RfD assessment is medium, both for the quality of the principal study and the sufficiency of the database. The principal study, assigned a confidence of medium, is a rat chronic oral study performed with relatively large group sizes, in which histopathological analyses on the known animal target tissue, the liver, were thoroughly performed. However, the discussion in IRIS stated that "available occupational studies, although limited, give no indication that the liver is a target organ in humans as a consequence of chronic exposure to low levels of chlordane" (http://www.epa.gov/iris/subst/0142.htm#umfinhal).

IRIS also reports that "recent evidence indicates that neurotoxicity, a known human endpoint in acute exposures, may be a relevant endpoint in chronic human exposures, and no chronic animals studies have examined neurotoxicity. Studies on pre-and postnatal animals indicating chlordane mimicry of sex-steroids raise reproductive concerns and no multigenerational reproductive studies, by any route, exist. Thus, there is some concern that the appropriate endpoints have not been examined adequately in the existing database." IRIS further states that "an area of scientific uncertainty in this assessment concerns the role of neurotoxicity, and possibly hematotoxicity, in chronic chlordane toxicity in humans." IRIS also notes that "another area of scientific uncertainty in this assessment concerns the toxicological significance of endocrine mimicry effects of chlordane. Toxicity data for this chemical include a study demonstrating biochemical and behavioral alterations consistent with technical chlordane (or its metabolites) mimicking male sex-steroids (Cassidy et al., 1994). That these effects could include reproductive behaviors is suggested in this study" (http://www.epa.gov/iris/subst/0142.htm#quaoral).

Studies on these endpoints would be of concern for children's health because accidental poisoning studies by chlordane in children have reported neuropsychiatric symptoms, which included learning disabilities, at an incidence four times that found in the general population according to the National Center for Health Statistics (Sherman, 1999). In20 poisoned children, 20 percent had hematological problems and an additional 15 percent had hematological dyscrasias which may be early indicators of leukemia and aplastic anemia (Sherman, 1999).

OEHHA PHG: 0.02 ppb (a safe dose of 1 x 10^{-5} mg/kg-day)

The PHG developed by OEHHA is 1 x 10^{-5} mg/kg-day, or 0.02 ppb in drinking water, based on a LOAEL of 0.1 mg/kg-day because of disruption of sex steroid-mediated behaviors in rat identified by Cassidy et al., 1994. The health-protective drinking water concentration for carcinogenic endpoints is calculated to be 0.03 ppb. The U.S. EPA drinking water unit risk is 1 x 10^{-5} per (µg/L) which translates into risk levels of 10^{-4} to 10^{-6} at concentrations of 3 ppb and 0.03 ppb, respectively (U.S. EPA, 1996).

Current Evaluation Results

Chlordane has been shown to have critical effects on two developing systems due to endocrine disruption. It adversely affects the developing immune system of mice (Spyker-Cranmer et al., 1982; Barnett et al, 1985a; Barnett et al., 1985b; Barnett et al.,

1990; Theus et al., 1992a; Theus et al., 1992b; Blyler et al., 1994), and it alters sex-mediated neurobehavioral endpoints (Cassidy et al., 1994). These effects on the developing endocrine and immune systems show an age-related susceptibility to chlordane. Adult animals exposed to similar or higher doses of chlordane did not show similar effects (Johnson et al., 1986; Barnett et al., 1990; Barnett, 1997).

An endocrine disruptor such as chlordane can act at the level of the hypothalamic-pituitary-adrenal (HPA) axis, disrupting the negative feedback loop between the brain and the immune system. Under normal physiological conditions, activation of the immune system stimulates the release of cytokines, which can then act on the HPA axis to trigger the release of corticosterone. However, if increased levels of corticosterone are released, due to the presence of endocrine disruptors, these high corticosterone levels produce immuno-suppression on virtually all levels of the immune system (Gaillard and Spinedi, 1998; Morale et al., 1995), including depression of the delayed-type hypersensitivity response (Okimura et al., 1986), suppression of granulocyte and macrophage migration (Mizobe et al., 1997), and inhibition of hematopoietic cytokines such as IL-3 and CFU-GM (Gaspar Elsas et al., 2000 and Mucha et al., 2000).

In addition, high levels of glucocorticoids can disrupt all aspects of the hypothalamic-pituitary-gonadal (HPG) axis, including reproductive behavior and the synthesis and release of sex steroids (Viau, 2002) and can interfere with the functioning of the hippocampus, the part of the brain responsible for learning and memory (Kim and Diamond, 2002). It has been shown that the release of adrenocorticotropic hormone (a hormone that stimulates the release of corticosterone) is associated with increased sexual excitation in male rats (Szechtman et al., 1974), and Bowman and colleagues (2001) demonstrated that female rats exposed to stress-induced increases in corticosterone levels showed altered spatial memory performance.

A key finding, suggesting that chlordane disrupts the HPA and HPG axis, was the observation that exposure of the dihybrid mice dams to 0.16 mg/kg-day of analytical reference standard chlordane (which has the same products as technical grade chlordane) from 0-18 days of gestation (Table 2.2.1) produced significantly elevated corticosterone in male and female offspring when they were assayed as adults at 100 and 400 days of age (Cranmer et al., 1984). This indicated a permanent (or long-lasting) effect on the offspring. This dose of technical grade chlordane also reduced metabolism of corticosterone in female BALBc mice and elevated resting plasma corticosterone in male mice at 100 days of age (Spyker-Cranmer et al., 1982). Corticosterone, like cortisol, is synthesized from progesterone by a series of hydroxylations. Testosterone is also synthesized from progesterone, and estradiol is synthesized from testosterone (Stryer, 1981). Chlordane can alter corticosterone levels, and corticosterone is an intermediate in the synthesis of steroids. By this mechanism, chlordane can affect the developing immune system, and it could permanently alter characteristic differences between males and females in non-reproductive and reproductive measures (such as body weight, development of sexual organs, circulating steroid levels, mating behavior, spatial abilities, activity level, or mixed function oxidase levels (Weiss, 2002). The endocrine

disruptive effect of chlordane appears to be corroborated by the study of Cassidy and colleagues (Cassidy et al., 1994) in which a dose of 0.1 mg/kg-day chlordane to the dam and then to the pups until postnatal day 80 caused sex steroid-mediated changes in gender-specific behaviors and functions (Cassidy et al., 1994). There was a dose-responsive decrease in plasma testosterone, which was significant at 5 and 0.5 mg/kg-day, but not significant at 0.1 mg/kg-day.

Table 2.2.1 Summary of Significant Studies on Chlordane

Reference	Protocol	Doses	Critical Effects
Spyker-Cranmer et al., 1982	Pregnant BALB/C mice were dosed until day 18 of gestation and pups nursed on their natural mothers until 21 days of age	0.16 and 8 mg/kg maternal body weight	Delayed Type Hypersensitivity (DTH) was significantly depressed at 8 mg/kg; and depressed but not significantly at 0.16 mg/kg
Cranmer et al., 1984	Pregnant F2 Dihybrid mice were dosed until day 18 of gestation and pups nursed on their natural mothers until 21 days of age.	0.16 and 8 mg/kg maternal body weight	Plasma corticosterone was significantly elevated at 101 days and 400 days in male mice whose mothers were dosed with 0.16 mg/kg-day. It was elevated in female mice at 400 days of age
Barnett et al., 1990	Pregnant BALB/C mice were dosed until day 18 of gestation and pups nursed on their natural mothers until 21 days of age	4 and 8 mg/kg maternal body weight	Hematopoietic stem cells (CFU-GM and CFU-S) in offspring were significantly decreased at 100 and 200 days of age. Adult animals treated with 8 mg/kg chlordane did not have any decrease or differ from controls.
Cassidy et al., 1994	Sprague-Dawley CD rats were dosed from Day 4 of gestation until Day 21 of lactation. Pups were dosed individually from post natal day (PND) 22 until PND 80.	0.1, 0.5, and 5 mg/kg maternal body weight	Females had significant improvements in spatial abilities in the Cincinnati Water Maze test at all doses, males exhibited dose-dependent increases in male-typical mating behavior, and both exhibited maximum response to auditory startle at 0.1 mg/kg when tested at 80 days.

The effects of chlordane on the developing immune system, and their persistence into adulthood, were demonstrated in a series of related studies using prenatal and postnatal exposure to chlordane (Spyker-Cranmer et al., 1982; Barnett et al, 1985a; Barnett et al., 1985b; Barnett et al., 1990; Blyler et al., 1994; Theus et al., 1992a; Theus et al., 1992b). The experimental protocol common to all the studies was to feed pregnant mice 0.3 mg of peanut butter which was spiked with technical chlordane to provide a maternal dose of 0.16 mg/kg, 4 mg/kg, 8 mg/kg or 16 mg/kg maternal body weight, although not all doses were used in the assay of each immune system parameter. The pups were allowed to

nurse through day 21. Assays of immune system parameters were performed at various postnatal days, ranging from day 42 to day 200, although not each immune system parameter was assayed at each postnatal time point. Chlordane is fat-soluble, having a log K_{ow} (octanol-water coefficient) of 5.16, so it should be readily transferred from plasma to milk. The total amount of chlordane reaching the pups was determined to be 3.5 mg/kg by analyzing chlordane and its metabolites in the conceptus and in pups at intervals during gestation and through the end of lactation (Theus et al., 1992)

Immune responses, such as delayed type hypersensitivity (DTH), were significantly depressed in offspring at 100 days of age after exposure in utero to a maternal dose of 8 mg/kg-day body weight. A maternal dose of 0.16 mg/kg-day also depressed DTH, although not significantly (Spyker-Cranmer et al., 1982). Pups received chlordane from 0-18 days of gestation, when the mother was dosed, and through 21 days of nursing, when dosing of the mother had ceased. Thus, the pup's exposure dose was actually lower than either 8 mg/kg-day or 0.16 mg/kg-day for its exposure duration.

A decreased DTH response occurs due to functional abnormalities in T lymphocytes, specifically the CD4 T_H1 helper cells. There are three kinds of effector T cells: cytotoxic CD8 T cells, which kill infected cells, and two kinds of CD4 T cells (T_H1, or T helper 1, and T_H2) with different functions (Parham, 2000). It is noteworthy that a decrease in the number of helper/inducer T cells is found in acquired immune deficiency (AIDS) disease, and this decrease is thought to allow infections such as Kaposi's sarcoma, Pneumocystis carinii pneumonia, and cytomegalovirus (CMV) retinitis (Lane and Fauci, 1985).

A critical effect on the developing immune system was a significant reduction in the number of granulocyte-monocyte committed stem cells (CFU-GM) and multipotential stem cells (CFU-S) in adult offspring (100 and 200 days of age) of pregnant mice exposed to 4 mg/kg and 8 mg/kg chlordane (Barnett et al., 1990). The bone marrow of offspring exposed to 4 or 8 mg/kg-day chlordane had 63 percent and 75 percent of control CFU-GM at 100 days of age, and at 50 percent and 77 percent at 200 days of age in offspring exposed to 4 mg/kg-day. The multipotential stem cells (CFU-S) were similarly depressed. Female and male offspring exposed prenatally to 8 mg/kg chlordane had 67 percent and 64 percent respectively of control CFU-S, while those exposed to 4 mg/kg-day chlordane had 78 percent and 87 percent of control CFU-S. At 200 days of age the bone marrow CFU-S in female offspring was almost unchanged, and that in males was still significantly reduced. This significant reduction in stem cells which could divide and differentiate into mature functional blood cells could produce life-threatening consequences. This decrease, as well a decrease in Interleukin-3 (IL-3) stem cells, was confirmed to be present at 42-49 postnatal days when specific recombinant growth factors were utilized (Blyler et al., 1994). IL-3 is a cytokine produced by T helper (T_H1 and T_H2) cells and it is a growth factor for multipotential progenitor hematopoietic cells (Parham, 2000). This toxicity endpoint is significant for humans because blood dyscrasias and bone marrow failures have been reported in people following accidental dermal or inhalation exposure to chlordane at unspecified dose levels (Infante et al., 1978; Klemmer et al., 1977; Furie and Trubowitz, 1976).

Clonogenic assays for hematopoietic progenitors have been used in clinical hematology for 30 years (Parent-Massin, 2001) and in research to predict adverse effects of drugs or toxicants, as the rapid rate of cell renewal and differentiation makes the hematopoietic system a susceptible target for xenobiotic toxicity. Xenobiotics that interfere with cell proliferation and differentiation can lead to "bone marrow failure." The two major groups of bone marrow failure are aplastic anemia, where the failure lies in the pluripotent stem cell (colony forming unit – stem cell or CFU-S), and single cytopenia, where the failure lies in the stem cell for one of the committed cell lines, such as the granulocyte/monocyte cell line, the CFU-GM (Parent-Massin, 2001). Most bone marrow failures are characterized by inadequate production of blood cells and, if severe, death of the organism results because existing numbers of stem cells have an inadequate ability to produce mature cells to provide oxygen (anemia), clot blood (thrombocytopenia), or to protect the organism from infection.

Endocrine disruptors such as chlordane can affect neuroendocrine/neurobehavioral endpoints, as well as immune endpoints. The studies of Cassidy and colleagues (Cassidy et al., 1994) confirmed that perinatal chlordane could mimic sex steroids and /or change their levels to masculinize sexually dimorphic functions and behaviors. They dosed pregnant rats with technical chlordane at 0.1 mg/kg, 0.5 mg/kg, and 5 mg/kg during gestation, and they dosed the offspring during 21 days of lactation and from postnatal day 22 to postnatal day 80. Female offspring committed fewer errors than controls in three assays of cognitive and spatial ability in the Cincinnati Water Maze test, appearing to behave more like males, and male offspring exhibited dose-dependent increases in male-typical mating behaviors. The differences in behavior compared to unexposed animals demonstrate that sexual differentiation of the neuroendocrine system has been altered by early life exposure to chlordane. The neuroendocrine-gonadal axis regulates the developmental organization and adult expression of behaviors critical for mammalian survival and reproduction (competitive aggression, exploration, and sexual and parental behaviors), so neurobehavioral alterations induced by endocrine disruptors may impact the survival and fitness of an individual in its environment (Palanza et al., 2002)

Recommendation

Based on studies that describe endocrine disruption and effects on the developing hematopoietic, immune and neuroendocrine systems in young animals, OEHHA recommends that a chRD be developed. The critical effects are alterations in characteristic behavior differences between males and female at doses of 0.1 mg/kg-day maternal body weight and 0.1 mg/kg-day pup weight (Cassidy et al., 1994), and disruption of the hematopoietic and immune systems at a maternal dose as low as 0.16 mg/kg-day maternal body weight (Cranmer et al., 1984).

OEHHA recommends that a non-cancer child-specific RD be calculated on the study by Cassidy and colleagues (Cassidy et al., 1994) that showed that a chlordane dosage of 0.1 mg/kg-day (to the pups, as well as the mother) disrupted sex hormone mediated

behaviors. Differences from control were significant at the lowest dose, indicating that 0.1 mg/kg dose is a LOAEL. Because these effects are indicative of endocrine disruption, it is possible that the hematopoietic/immune effects described in the other studies may also occur at this low dose.

Calculation of the non-cancer child-specific RD for chlordane is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.1 \text{ mg/kg-day}}{3000} = 3.33 \times 10^{-5} \text{ mg/kg-day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level (Cassidy et al, 1994)

UF = Uncertainty factors of 3000 (10 for LOAEL to NOAEL, 10 for interspecies extrapolation, 10 for human variability, and 3 for inadequate database for hematotoxicity, immunotoxicity, neurotoxicity, and the lack of a valid developmental study).

Accordingly, OEHHA is proposing a non-cancer chRD of 3.3×10^{-5} mg/kg-day for chlordane.

Uncertainty and Modifying Factors

OEHHA has applied the additional uncertainty factor 3 for inadequacies in the database for each of three endpoints, hematotoxicity, neurotoxicity, and reproductive toxicity that data suggest may be of concern to human children. This uncertainty factor has been applied in accordance with U.S. EPA (1994) and Renwick et al., (2000), that it is appropriate "if a valid developmental toxicity study was not performed", or "if the study did not examine all developmental endpoints". In the discussion of chlordane on the IRIS database, U.S. EPA noted that "studies on pre-and postnatal animals indicating chlordane mimicry of sex steroids raise reproductive concerns and no multigenerational reproductive studies, by any route, exist. Thus, there is some concern that the appropriate endpoints have not been examined adequately in the existing database" (http://www.epa.gov/iris/subst/0142.htm#umfinhal).

The U.S. EPA RfD was based on hepatic necrosis in mice, even though the discussion on the IRIS database noted that "occupational studies, although limited, give no indication that the liver is a target organ in humans as a consequence of chronic exposure to low levels of chlordane." U.S. EPA reduced confidence in their RfD noting that "an area of scientific uncertainty concerns the role of neurotoxicity and possibly hematotoxicity in chronic chlordane toxicity in humans" (http://www.epa.gov/iris/subst/0142.htm#umfinhal). Neurotoxicity and hematotoxicity

have been reported as principal endpoints of acute chlordane toxicity in both experimentally poisoned animals and accidentally poisoned humans (Grutsch and Khasawinah, 1991; Fleming and Timmeny, 1993). The uncertainty increases when considering exposure of children, rather than adults, because these organ systems are undergoing critical development during childhood.

The reduced numbers of hematopoietic stem cells in offspring, after they had reached maturity, from exposure to a 4 mg/kg-day maternal dose (Barnett et al., 1990a), provides low confidence that a LOAEL which produced minimally significant adverse hematological effects was identified. Immune system toxicity from chlordane is a concern because a report of outcomes from exposure of 20 children to chlordane from pesticide applications noted that 20 percent had hematological problems and an additional 15 percent had hematological dyscrasias (Sherman, 1999). Blood cell dyscrasias are a concern to clinicians because they may later manifest themselves as leukemias and aplastic anemias. The outcome of reduced numbers of stem cells can be bone marrow failure.

The database on chlordane toxicity to children is also considered inadequate because no animal studies have adequately assayed neurotoxicity due to low chlordane exposure concentrations, and there are case reports of human neurotoxicity (Kilburn and Thornton, 1995; Kilburn, 1997). Al-Hachim and Al-Baker (1973) reported that when pregnant mice were exposed to 1 mg/kg-day technical chlordane for only seven consecutive days the pups had poor learning ability or altered motivation in the assay for conditioned avoidance response, raised seizure threshold, and increased exploratory activity. Reports of accidental human exposure to termiticides have resulted in neurobehavioral impairments in adults (Kilburn and Thornton, 1995; Kilburn, 1997) and 70 percent of 20 child patients exposed to chlordane had neuropsychiatric symptoms, which included learning disabilities at an incidence four times that found in the general population, according to the National Center for Health Statistics (Sherman, 1999). School-age children were reported to develop new problems: headaches, visual difficulties, hyperactivity, learning disabilities, frequent ear-nose-throat and chest problems, and gastrointestinal disturbances (Sherman, 1999).

The Food Quality Protection Act required a 10 fold safety factor be applied "for infants and children" for pesticide risk assessments "to take into account…completeness of the data with respect to … toxicity" and OEHHA utilized the 10 fold factor in creating a public health goal (PHG) for chlordane. However, U.S. EPA has been limiting the composite factor to 3,000 when human-equivalent doses are used (U.S. EPA, 1994). As the low dose in the Cassidy et al. (1994) study, which forms the basis for the child-specific RfD, was based on serum levels found in the United States at the 99th percentile (i.e. 1% of the U.S. values are higher), OEHHA decided to utilize only a 3-fold modifying factor to account for the inadequacies in the database.

Additional Comments/Studies:

The experiments from Barnett and colleagues (Spyker-Cranmer et al., 1982; Barnett et al, 1985a; Barnett et al., 1985b; Barnett et al., 1990; Theus et al., 1992a; Theus et al., 1992b; Blyler et al., 1994) support an equivalent or lower dose than the one derived from the Cassidy et al., 1994 study. The experimental protocol of Barnett and colleagues differed from that of Cassidy et al., 1994, in that the pup was not individually dosed. A maternal dose of 8 mg/kg-day was reported to produce a *total* dose of 3.5 mg/kg of chlordane in the pup (Theus et al., 1992). If an equal fraction of 3.5 mg/kg dose were delivered each day of the 18 days of gestation, when the mother was dosed, and the 21 days of lactation, when the mother was not dosed, the pup would receive 0.09 mg/kg-day over the 39 day period. This estimated pup dose is very close to the 0.1 mg/kg-day dose that Cassidy et al. (1994) gave to dams, *and* to pups following weaning and until sacrifice on day 80.

The experiment of Cranmer et al. (1984) suggests that the LOAEL could be lower than 0.1 mg/kg-day. In this study, the dams were dosed with either 0.16 mg/kg-day or 8 mg/kg-day chlordane during gestation. As noted above, the maternal dose of 8/mg/kg-day was shown to produce a total dose of 3.5 mg/kg in the pups when the concentration of chlordane metabolites analyzed on successive days during the 39 days of gestation and lactation were totaled. The average daily dose to the pup was 0.09 mg/kg-day. If the toxicokinetics from the 0.16 mg/kg-day maternal dose is proportional to that from the 8 mg/kg-day maternal dose, then the pups of a dam dosed with 0.16 mg/kg-day (Cranmer et al., 1984) would receive 0.0018 mg/kg-day. Emerging understanding of the hypothalamic-pituitary-adrenal (HPA) axis substantiates the possibility that a low dose may impair the developing immune system.

2.3 Heptachlor/Heptachlor Epoxide

Heptachlor (heptachlorodicyclopentadiene) was used primarily as an agricultural insecticide from 1952 to 1976, as a narcissus bulb and seed treatment and insecticide for fire ant control on pineapple crops until 1976, and as a treatment for subterranean termites until 1987 (Fendick et al., 1990). In 1985, heptachlor alone or in combination with chlordane, accounted for 60-65 percent of the termiticides used in the U.S. (EPA 1987 – see Fendick et al.). In 1987, the EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) classified heptachlor as a priority Group 1 Hazardous Substance, making Superfund money available for cleanup of heptachlor-contaminated sites.

Technical heptachlor contains heptachlor plus related reaction products in approximately a 5:2 ratio (Fendick et al., 1990). Heptachlor is a moderately persistent compound (Ware et al., 1990). In the soil it undergoes multiple transformation and degradation reactions by at least three pathways: epoxidation, hydrolysis, and dechlorination. Epoxidation generates the more persistent and bioaccumulative metabolite, heptachlor epoxide, while hydrolysis is a detoxification reaction (Fendick et al., 1990). U.S. EPA has considered heptachlor and heptachlor epoxide two separate chemicals, and it has established separate RfDs, probably because heptachlor epoxide absorbs strongly to soil and is extremely resistant to biodegradation (Hazardous Substances Databank, http://toxnet nlm.gov), persisting in soils for a long time (Ware, 1988).

OEHHA included heptachlor/heptachlor epoxide in the "Compilation of Chemicals Potentially Found at School Sites" because it has been targeted by federal and state agencies as a chemical that may present environmental health risks. Heptachlor appears on all but two of the chemical compilations that OEHHA has selected to identify chemicals that may be found at school sites. These compilations include:

- Soil contaminants identified at potential school sites in environmental investigations reviewed by the Department of Toxic Substances Control
- Toxic Air Contaminants (TACs) in California identified by OEHHA
- Analytes in the U.S. EPA National Health Exposure Assessment Study (NHEXAS)

OEHHA also included heptachlor/heptachlor epoxide in the compilation of "Candidate Chemicals Based on Critical Health Effects" because heptachlor epoxide is on the Proposition 65 Developmental and Reproductive Toxicant List, and a survey of recent scientific literature indicated that heptachlor and heptachlor epoxide are toxic to organ systems that are developing in children. These organ systems are the immune, nervous, endocrine, and male and female reproductive systems (Brucker-Davis, 1998; DeRosa et al., 1998; Moser et al., 2001; Nicolopoulou-Stamati et al., 2001; Rani et al., 1995; Smialowicz et al., 2001; Voccia et al., 1999). Heptachlor and heptachlor epoxide were also reported to produce cancer (Zahm et al., 1998 and http://toxnet.nlm.nih.gov).

Existing Health Guidance Values

U.S. EPA Carcinogen Slope Factor: 4.5 per mg/kg-day

Heptachlor is classified by EPA as a B2, probable human carcinogen, based on several studies. Davis (1965) fed groups of 100 male and 100 female C3H mice diets with 0 or 10 ppm heptachlor (purity not specified) for 2 years. Survival was low, with 50 percent of the controls and 30 percent of the treated mice surviving until the end of the experiment. A two-fold increase in benign liver lesions over the controls was reported. After a histologic reevaluation, Reuber (as cited in Epstein, 1976), as well as four other pathologists, remarked a statistically significant increase in liver carcinomas in the treated male (64/87) and female (57/78) groups by comparison to controls (22/73 and 2/53 for males and females, respectively). The NCI (1977) reported a significant dose-related increase of hepatocellular carcinomas in male and female B6C3F1 mice.

U.S. EPA RfD: 5×10^{-4} mg/kg-day

The current oral RfD for heptachlor given by U.S. EPA in 1991 is 5×10^{-4} mg/kg-day (http://toxnet.nlm.nih.gov). This value was derived from a three ppm dietary NOAEL in a two-year rat feeding study where the critical effect was liver weight increase (Velsicol Chemical, 1955, cited by U.S. EPA http://toxnet.nlm.nih.gov/). The LOAEL in this study was 5 ppm or 0.25 mg/kg-day and an uncertainty factor of 300 was employed. EPA reports that there is low confidence that this RfD is accurate because the principal study is of low quality; the database on chronic toxicity is incomplete. There are no teratology, reproductive, or studies in young animals.

An RfD for heptachlor epoxide was based on a study in which adolescent dogs were fed heptachlor epoxide for 60 weeks (Dow Chemical Company, 1954). The LOAEL of 0.5 ppm (0.0125 mg/kg-day) was based on an increased liver-to-body weight ratio in both males and females as a critical effect (Dow Chemical Co., 1958, cited in U.S. EPA IRIS online file, http://toxnet.nlm.nih.gov/). An uncertainty factor of 1000 was employed. EPA indicates there is low confidence that the RfD is accurate because the principal study is of low quality, and the chronic toxicity studies are of low quality. There were no rat or rabbit teratology studies.

OEHHA PHG: 8 ppt (a safe dose of 1×10^{-4} mg/kg-day)

OEHHA staff prepared a Public Health Goal (PHG) for heptachlor of 8×10^{-6} mg/L drinking water, based on a cancer slope factor of 4.1 mg/kg-day $^{-1}$ and a 1×10^{-6} cancer risk (OEHHA, 1999b). Heptachlor exposure produced a dose-related increase in the incidence of hepatocellular carcinoma in male and female B6C3F1 mice (NCI, 1977) and hepatocellular carcinoma in male and female C3H mice (Davis, 1965).

OEHHA (1999) considered two studies when non-cancer effects were reviewed. The first was the two-year rat feeding study where the critical effect was liver weight increase that was used by EPA when it last revised the heptachlor RfD (Velsicol Chemical, 1955, cited by U.S. EPA, http://toxnet.nlm.nih.gov). Uncertainty factors of 10 for interspecies variability and 10 for interindividual variability were used. The second study (Cassidy et al., 1994) reflected the recent concern about the endocrine disruption effects of chlorinated cyclodiene and other chlorinated pesticides. The critical effect in this study was the alteration of sex steroid-mediated behaviors by prenatal and early-in-life exposure to 0.1mg/kg/day technical chlordane which contains 10percent heptachlor. In this calculation, the uncertainty factors are: LOAEL to NOAEL extrapolation (10), interspecies variability (10), and interindividual variability (10), resulting in a "safe" non-cancer human dose of 1×10^{-4} mg/kg/day

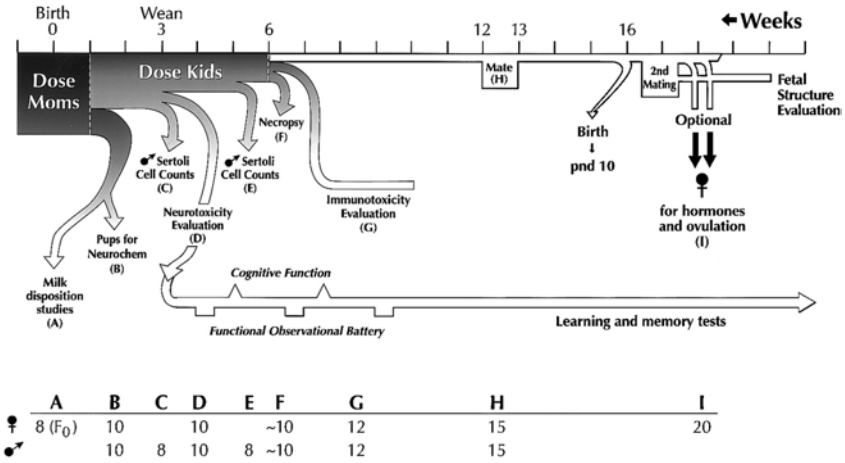
Current Evaluation Results

The effects of heptachlor that are specific for children are its disturbance of the development of the endocrine system and of the organs that respond to endocrine signals when exposure occurs during prenatal and/or early postnatal life (Colborn, 1993). These effects are permanent.

Contamination of the commercial milk supply of Oahu, Hawaii, with heptachlor for 15 months, from 1981 to 1982, and the subsequent finding of heptachlor epoxide in human milk, prompted new studies on rats to look for possible effects of heptachlor and its persistent primary metabolite at the concentrations to which children were exposed. The Hawaii Heptachlor Research and Education Foundation (HHREF) cosponsored these studies with the U.S. EPA and NIEHS in order to evaluate many aspects of the impact of heptachlor exposure during the perinatal/juvenile period of development, using a broad battery of tests of immune and reproductive system function.

The doses (0, 0.3, 3, or 30 mg/kg-day of 99% pure heptachlor) employed were adjusted so that the low dose gave milk values of heptachlor epoxide that approximated the 95th percentile of human milk heptachlor and heptachlor epoxide values in Oahu, Hawaii in 1981 (Baker et al., 1991; Siegel, 1988 in Smialowicz et al., 2001). The period of exposure was designed to approximate the last trimester of pregnancy through 18 years of age in humans. The experimental design for the studies of endocrine disrupting effects on immune, neurobehavioral, and reproductive is given in Figure 2.3.1.

Figure 2.3.1 Experimental Design



	A	B	C	D	E	F	G	H	I
♀	8 (F_0)	10		10		~10	12	15	20
♂		10	8	10	8	~10	12	15	

Figure taken from Moser et al., 2001 and Smialowicz et al., 2001)

The results of one subset of this study (Smialowicz et al., 2001) indicated that exposure of rats to heptachlor during the last trimester of gestation through puberty adversely affects adult functioning of the immune system by suppressing the primary IgM and secondary IgG antibody response in male offspring. Rats were exposed to 0, 0.3, 3, or 30 mg/kg-day from gestation day 12 to postnatal day 7, followed by direct dosing through postnatal day 42. The LOAEL was identified as 0.3 mg/kg-day because the primary IgM antibody response to Sheep Red Blood Cells (SRBCs), as measured by enzyme linked immunosorbent assay (ELISA) was suppressed at 8 weeks of age and at 21 weeks of age. At the same dose, the secondary IgG response was also suppressed at 25 weeks of age. These responses require three major immune cell types: macrophages, the CD4+ T-helper cells, and B cells. Alterations in or dysfunction of any of these cells and cell interactions can result in aberrant antibody production (Luster et al., 1988 in Smialowicz, 2001). The suppression of these T cell-dependent antibody responses persisted through the first six months of life at all doses employed, including the lowest dose, 0.3 mg/kg-day, which was administered through 6 weeks of age.

The response to SRBC is one of the most sensitive functional parameters in animals exposed to immunosuppressants (Luster et al., 1992). Consequently, it is included in the battery of tests required by the Federal Insecticide, Fungicide, and Rodenticide (FIFRA) guidelines for detection of immunosuppressants (Smialowicz, 2001). In both animals and humans, T-cell dependent responses are involved in protection against viral, bacterial, and parasitic infections (Blanden, 1974 in Smialowicz, 2001). Consequently, the suppression of the primary IgM and secondary IgG antibody responses suggests potential increased susceptibility to infectious diseases.

In another subset of rats from the same large study, heptachlor produced significant differences in tests for cognitive abilities that are associated with the development of neuroendocrine pathways (Moser et al., 2001). Rats were evaluated for neurological and behavioral alterations using a functional observational battery (FOB), an automated measure of motor activity, passive avoidance, and a Morris water maze test (Moser et al., 2001). Rats dosed prenatally and postnatally until day 21 had changes in activity measures, but those in which dosing continued until day 42 had alterations in autonomic, neuromuscular, and excitability measures. The most pronounced effects of heptachlor occurred in rats treated until day 42 and tested with the Morris water maze test. The Morris water maze test (Morris, 1984) was devised to resolve theoretical controversies about the basis of spatial and working memory. Normal rats learn very quickly to swim directly towards a platform from any starting position at the circumference of a pool. The accurate directionality of their escape behavior provide evidence that the rats escape by learning the position of the platform relative to distal cues. Thus, their performance can be compared to those of animals exposed to potential neurotoxins to assay spatial learning and memory (Morris, 1984).

Heptachlor exposure slowed acquisition of the spatial task and impaired recall during probe trials: the treated male rats at all dose levels did random searching for the platform, rather than developing an efficient search strategy. Working memory, which was assayed by requiring the rats to learn a new position for the platform each day, was significantly decreased in the low dose (0.03 mg/kg-day) male rats which had been dosed with heptachlor prenatally and postnatally until Day 21. The escape latency (mean time to find the new location) was 27.9 seconds compared to 20.5 seconds in control (Smialowicz, et al., 2001).

Cyclodiene pesticides bind to the chloride channel portion of the receptor for the neurotransmitter gamma aminobutyric acid $(GABA)_A$, block the inhibitory actions of and thus affect a variety of neurological functions in both adult and young animals (Abalis et al., 1986; Cole and Casida, 1986; Gant et al., 1987 in Moser, 2001). Acute actions of cyclodiene pesticides include excitation, hyperstimulation, and convulsions (Cole and Casida, 1986; Fendick et al., 1990). In young mammals, the development of the nervous system is quite protracted, and specific processes of migration, proliferation, and differentiation occur from gestation throughout childhood and into adolescence. These processes occur in sequence, so disturbance of earlier processes can disrupt later developmental events. Cyclodiene insecticides alter expression of the $GABA_A$ receptor. Since the neurotransmitter, GABA, influences development of serotonergic, dopaminergic, and cholinergic neurotransmitter systems, cyclodiene pesticides may produce long-lasting alterations in brain function (Lauder et al., 1998 in Smialowicz et al., 2001).

Recommendation

Heptachlor:

The experiments of Smialowicz et al. (2001) and Moser et al. (2001) describe several critical effects in young male rats at a LOAEL of 0.03 mg/kg-day heptachlor during the last half of gestation and the first 21 or 42 postnatal days. The most significant effects include suppression of the primary IgM and secondary IgG antibody response (Smialowicz et al., 2001), and decreased performance on measures of cognitive function, such as impaired recall (Moser et al., 2001).

Technical grade heptachlor and chlordane are mixtures of pure compound plus related reaction products (Ware, 1990). The PHG developed by OEHHA in 1999 utilized a study (Cassidy et al., 1994) that described a disruption of sex-steroid mediated behaviors in female mice at a dose of 0.01 mg/kg-day of technical grade chlordane, which contains 10 percent heptachlor. More recent studies (Smialowicz et al., 2001, Moser et al, 2001), which used heptachlor of 99 percent purity, have allowed OEHHA staff to develop the chRD for heptachlor without the ambiguity associated with testing a mixture.

Calculation of the non-cancer chRD is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.03 \text{ mg/kg-day}}{1000} = 3.0 \times 10^{-5} \text{ mg/kg-day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level from Smialowicz et al., 2001 and Moser et al., 2001

UF = Uncertainty factor of 1000 (10 for LOAEL to NOAEL, 10 for inter-species extrapolation, 10 for human variability)

Accordingly, OEHHA is proposing a non-cancer chRD of 3.0×10^{-5} mg/kg-day for heptachlor.

Heptachlor Epoxide:

Heptachlor has not been used since 1987 when its use was restricted. Heptachlor in the soil undergoes multiple transformation and degradation reactions, and epoxidation generates the more persistent and bioaccumulative metabolite, heptachlor epoxide (Fendick et al., 1990), so children at school sites may be exposed to heptachlor epoxide.

The principal study used by U.S. EPA to calculate an RfD (http://toxnet.nlm.nih.gov) was a 60-week dog feeding study (Dow Chemical Co., 1958) in which the LOAEL was an increased liver-to-body weight ratio. Although liver-to-body weight ratio is not a child-

specific endpoint, the exposure period began in adolescence and continued into young adulthood.

Since adolescent animals were exposed, OEHHA has decided to utilize the same study and the same uncertainty factors to calculate a non-cancer child-specific RD as the U.S. EPA RfD:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.0125 \text{ mg/kg-day}}{1000} = 1.3 \times 10^{-5} \text{ mg/kg-day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level from Dow Chemical Co, 1958

UF = Uncertainty factor of 1000 (10 for LOAEL to NOAEL, 10 for inter-species extrapolation, 10 for human variability)

Accordingly, OEHHA is proposing a non-cancer chRD of 1.3×10^{-5} mg/kg-day for heptachlor epoxide.

2.4 Methoxychlor

Methoxychlor, 2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane, is structurally related to DDT. Because of its lower toxicity and bioaccumulation potential, methoxychlor became an attractive replacement of DDT (ATSDR, 1994). It was registered as an insecticide against a wide range of pests, including houseflies, mosquitoes, cockroaches, and various arthropods commonly found on fields crops, vegetables, fruits, stored grain, livestock, and domestic pets.

While DTSC's school site review efforts continue, methoxychlor has already been detected at a school site. Recent studies show that demethylated metabolites of methoxychlor are an endocrine disruptor. Accordingly, OEHHA believes it is important to further review methoxychlor pursuant to Health and Safety Code Section 901(g). As endocrine disruptors, methoxychlor metabolites may have adverse effects on different developing organ systems. These chemicals may disrupt the development and functioning of the female reproductive system, and the brain, and the male reproductive system (vom Saal et al., 1983, 1997; Nonneman et al., 1992; Hess et al., 1997). As such, the effect of methoxychlor or its metabolites could affect the development of school children. Moreover, earlier studies may have inadequately characterized the dose-response relationship of methoxychlor (NTP, 2001). In reviewing environmental estrogens, the NTP Peer Review Subpanel found that overall the classic estrogenic activity of methoxychlor was limited to doses greater than 5 mg/kg-day because testing at lower doses had not been incorporated into the experimental design. An updated review of the pertinent literature is necessary to ensure that the appropriate LOAEL or NOAEL will be considered in setting a child-specific guidance value.

Pertinent Guidance Values

U.S. EPA RfD: 0.005 mg/kg-day

U.S. EPA used the 1986 Kincaid Enterprises, Inc. study to establish its RfD. Young adult female New Zealand White rabbits were assigned into 3 dose groups of 17 animals each, 5.01, 35.5, and 251.0 mg/kg-day, and a control group (a total of 68 animals). The females were artificially inseminated and the day of insemination considered as gestation day 0. All animals were dosed from days 7 through 19 of gestation. All surviving dams were sacrificed on gestation day 29.

Maternal toxicity was observed as excessive loss of litters (abortions) and statistically significant decreases in body weight in the mid- and high-dose groups, and the deaths in the high dose group. No specific toxicity was noted in the low dose (5.01 mg/kg-day), which was deemed to be the NOAEL.

An uncertainty factor of 1000 was applied to the NOAEL in developing the RfD; of which 100 was used to account for the inter-and intra-species differences and an

additional 10 was used to account for the poor quality of the critical study and for the incomplete database on chronic toxicity.

OEHHA PHG: 0.03 mg/L (a safe dose of 0.005 mg/kg-day)

In reviewing literature for the purpose of establishing a Public Health Goal (PHG) for methoxychlor in drinking water (OEHHA, 1999c), OEHHA found the chemical to be negative in several mutagenicity tests. However, a positive test was reported for the induction of forward mutations in the mouse lymphoma assay. Large doses of methoxychlor decreased locomotor activity and caused tremors. Reproductive effects have been caused by the estrogenic activity of the o-demethylated metabolites of methoxychlor. These metabolites also bind estrogen receptors in animal and human tissues.

OEHHA identified the investigation by Chapin et al. (1997) as the most relevant study for use in developing a PHG for methoxychlor in drinking water. The investigation focused on effects of perinatal methoxychlor exposure on adult rats' nervous, immune, and reproductive system function. Dams were dosed orally at gestation day 14 through postnatal day (pnd) 7 and then pups were directly dosed at pnd 7 through pnd 42 at dosages of 0, 5, 50, and 150 mg/kg-day. Critical effects included a reduction in serum FSH, ovary weight and uterine weight at all dosages. The findings suggested that methoxychlor, as an exogenous estrogenic agent, had interfered with the normal programming of the ovarian-pituitary axis.

Applying the LOAEL of 5 mg/kg/day and an uncertainty factor of 1000 (10 each for inter-species extrapolation, intra-human variability, and LOAEL to NOAEL extrapolation), OEHHA calculated a safe dose of 0.005 mg/kg-day. This in turn was used to derive a PHG of 0.03 mg/L.

Current Evaluation Results

Stoker et al. (1999) investigated the effect of perinatal exposure of methoxychlor on the prostate of adult rat. The study showed that a perinatal dose of 50 mg/kg methoxychlor to the dam only from gestation day 18 to postnatal day 5 resulted in offspring with increased lateral prostate weight and inflammation at 90 days of age.

Welshons et al. (1999) reported increases in adult prostate size in mice from fetal exposure to methoxychlor. Females were dosed from day 11 to day 17 of pregnancy at 20 or 2000 μg/kg maternal body weight per day. Pups were weaned on postnatal day 23. When males reached 8.5 months old (adult), a randomly selected male from each litter was individually housed for 4 weeks to eliminate any effects of having been housed with other males before the selected male was sacrificed for various examinations. Prostatic weights were significantly increased in the 20 and 2000 μg/kg groups.

Table 2.4.1 Effects of Methoxychlor on Prostate Weight

	Prostate (mg)
Control	40.0±3.0
Methoxychlor (20 μg/kg)	64.5±3.7
Methoxychlor (2000 μg/kg)	60.3±4.1

The finding is not surprising as the prostate contains both androgen and estrogen receptors (Kumar et al., 1995) and it has been observed that estrogen can stimulate the growth of the stromal compartment of the prostate (Ekman, 2000). The study also illustrates the irreversible impact of endocrine disruptors during development.

vom Saal et al. (1995) discussed evidence that during fetal life, hormones have marked effects on subsequent behaviors. Male mice are particularly active in urine-marking behavior to indicate their social status. Urine marking was used as the end point to measure the effect of methoxychlor. Females received 0, 1, 10, 100, 1000, or 5000 μg/d from day 11 to day 17 of pregnancy. Two males from each litter were randomly selected when they were 60 days old and housed individually for four weeks to eliminate any effects of having been housed with other males. Urine-marking tests were conducted for one hour in clean cages with the floor lined by a sheet of Whatman No. 2 filter paper. The filter paper was then removed and discrete urine marks (which fluoresce under UV light) deposited on it were counted. The lowest dose (1 μg/day or 20 μg/kg-day based on 0.05 kg maternal weight) of methoxychlor significantly increased urine-marking behavior in male offspring.

Recommendation

OEHHA recommends that a chRD for methoxychlor be developed based on the data from Welshons et al. (1999) and vom Saal et al. (1995). While the exposure period used to demonstrate the significant effect on the prostate or neurobehavior was in the fetal period, OEHHA feels that both the prostate and neurobehavioral data (territorial marking) are applicable for school age children. The human prostate development is biphasic, with much of the growth occurring at puberty. It is small (weighs about 2 g) in childhood and undergoes exponential growth to about 20 g at puberty (Hayward et al., 2000). On the other hand, the process of brain development and maturation continues into adolescence (Rice et al., 2000). These systems remain vulnerable during the K-12 schooling period. Therefore, the Welshons and vom Saal data are an appropriate basis for evaluating hazards at schools.

OEHHA has also considered the appropriateness of using the maternal dose to calculate the chRD. OEHHA finds that methoxychlor crosses the placenta and partitions into the

lipids of milk (OEHHA, 1999). It is likely that the corresponding pup dose is higher on a per kilogram body weight basis. However, the demethylated (phenolic) metabolites rather than methoxychlor were shown to be the active species that displayed the endocrine disruption potential. The polar metabolites would not cross the placenta effectively. Additionally, methoxychlor that crosses the placenta would not be metabolized effectively by the pup whose P-450 enzymes are not fully developed. Thus, it would not be too conservative to use the maternal dose in this case to calculate the chRD.

Calculation of the non-cancer chRD for methoxychlor is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{20\ \mu\text{g/kg-day}}{1000} = 0.02\ \mu\text{g/kg-day}$$

Where,

LOAEL= Lowest-observed-adverse-effect-level based on Welshons et al. (1999) and vom Saal et al. (1995)

UF= Uncertainty factor of 1000 (10 for inter-species extrapolation, 10 for intra-human variability, and 10 for LOAEL to NOAEL extrapolation)

Accordingly, OEHHA is proposing a non-cancer chRD of 0.02 µg/kg-day for methoxychlor to be used in school-site risk assessment.

2.5 Nickel

Nickel, an important industrial metal, comprises 0.008 percent of the earth's crust (Duke, 1980 as cited in ATSDR, 1997). The production, use, and disposal of nickel have led to its mobilization in the environment and human exposure. Nickel is used in aircraft frames, jet engines, gas turbines, and turbosuperchargers, boats, hulls, propellers, and pumps (OEHHA, 2001). Nickel alloys are used in pumps and pipes to resist corrosion in petro-chemical industries. In addition, nickel is used in making coins and jewelry; as catalysts; and in magnets, batteries, and color pigment. Nationwide in 2000, 651,000 pounds of nickel were emitted into the air (with nickel plating operations as a major source of emission), 30,000 pounds were discharged into surface water, 17,000 pounds were injected underground, 2,032,000 pounds were disposed of onsite, and 8,700,000 pounds were disposed of offsite (U.S. EPA, TRI2000).

Nickel was selected for further evaluation pursuant to Health and Safety Code Section 901(g) because it meets both criteria for selection identified in OEHHA's 2002 report (OEHHA, 2002):

- DTSC reported the presence of nickel at two percent of the potential school sites evaluated to date. ARB reported its occurrence in California air (OEHHA, 2002). In addition, U.S. EPA and ARB/DHS have deemed nickel as a chemical of interest in their NHEXAS and Portable Classroom Study, respectively.

- OEHHA (2001) found a number of studies concerning the reproductive effects of nickel compounds. Nickel also adversely affected the immune functions in animals. The administration of nickel to rats increased the concentration of the metal in the hypothalamus and pituitary and inhibited prolactin secretion.

Pertinent Guidance Values

U.S. EPA RfD: 0.02 mg/kg-day

U.S. EPA's RfD is based primarily on the results of a two-year feeding study using rats given 0, 100, 1000 or 2500 ppm nickel (estimated as 0, 5, 50 and 125 mg Ni/kg bw) in the diet (Ambrose et al. 1976). In the 1000 and 2500 ppm groups (50 and 125 mg Ni/kg bw, respectively) body weights were significantly decreased compared with controls and the females had significantly higher heart-to-body weight ratios and lower liver-to-body weight ratios than controls. Since no significant effects were reported at 100 ppm (5 mg Ni/kg bw), this dose was a NOAEL. In this study, two-year survival was poor, particularly in control rats of both sexes (44 of 50 died), raising some concern about the interpretation of the results of this study. A subchronic study conducted by American Biogenics Corp. (ABC, 1986) also found 5 mg/kg-day to be a NOAEL, which supported the Ambrose et al. (1976) chronic NOAEL of 5 mg/kg-day.

An uncertainty factor (UF) of 300 (10 for interspecies extrapolation, 10 to protect sensitive populations, and 3 to account for inadequacies in the reproductive studies) was applied to the NOAEL of 5 mg/kg-day to compute an RfD of 0.02 mg/kg-day.

OEHHA PHG: 11.8 µg/L (a safe dose of 1.1 x 10^{-3} mg/kg-day)

OEHHA (2001) established a Public Health Goal (PHG) for nickel in drinking water that is based on three reproductive studies in rats (Smith et al., 1993; Springborn Laboratories, 2000a, b). In the Smith study 61-64 day old female rats (at puberty) were dosed at 0, 1.3, 6.8, or 31.6 mg/kg-day for 11 weeks prior to mating and then continuously during two sequential gestation and lactation periods. Breeder males were unexposed. The proportion of dead pups per litter was significantly increased in the 31.6 mg/kg-day group in both breedings and also in the 1.3 mg/kg-day group in the second breeding. Thus, 1.3 mg/kg-day was considered the LOAEL for this study.

The first Springborn report (Springborn Laboratories, 2000a) summarized a one-generation reproduction range-finding study in rats. 102 day-old animals (at sexual maturity) were dosed at 0, 10, 20, 30, 50, or 75 mg nickel sulfate hexahydrate/kg-day for two weeks prior to mating. OEHHA observed significant pup mortality at the lowest dose (10 mg nickel sulfate hexahydrate/kg-day or equivalent to 2.2 mg nickel/kg-day) and deemed it as the LOAEL for this study.

Following the range-finding study, Springborn Laboratories (2000b) conducted a two-generation reproduction study. Nickel sulfate hexahydrate was administered at 0, 1, 2.5, 5, or 10 mg/kg-day. Dosing of the F_0 animals began at 10 weeks prior to mating and dosing of the F_1 rats began on postpartum day 22 (just after weaning, at a young age). For both generations, daily dosing of the dams was continued until lactation day 21. In this two-generation study, no adverse effects were observed even at the highest dose, 10 mg/kg-day (2.2 mg nickel/kg-day).

In reviewing these three studies in totality, OEHHA concluded that the 1.1 mg nickel/kg-day (5 mg nickel sulfate hexahydrate/kg-day) dose in the two-generation study was the appropriate NOAEL for use in calculating the PHG. It represents the highest NOAEL that is lower than the LOAEL from either the Smith, or Springborn range-finding, study.

OEHHA applied this NOAEL in conjunction with an uncertainty factor of 1000 (10 for inter-species extrapolation, 10 to account for human variability, and 10 for database deficiencies for carcinogenic effect via oral route) for calculating a safe dose of 1.1 µg/kg-day. The safe dose was in turn used to derive the PHG.

Current Evaluation Results

Nickel has been cited in the PHG report as having adverse effects on several sensitive organ systems that are undergoing development in school children (OEHHA, 2001). For

example, it affected the hypothalamus-pituitary axis and inhibited prolactin secretion; it reduced a variety of T-lymphocytes and natural killer cell-mediated immune functions; and it impacted the reproductive system and viability of offspring. Against this background, OEHHA targeted the literature search using the criteria outlined in the Introduction Section. We came up with a list of 18 references; all of which were qualitative studies and thus not usable in the context of the current task.

OEHHA modified its strategy, which stipulated a broad-based literature search. A total of 18,410 references were compiled. These references and their abstracts were put into a Procite database. The database, in turn, was queried in an attempt to identify quantitative studies with nickel doses in the range, or below that, of 1.1 mg/kg-day. The purpose is to run another check that we have identified the "lowest" LOAEL or NOAEL during the PHG review. The results support that conclusion.

Recommendation

The current broad-based literature search has not identified data to suggest that the NOAEL should be changed from that used as the basis for the PHG. The PHG NOAEL addresses the reproductive end point that is one of the targeted organ systems for this review, and the exposure time and duration of rats stipulated in the Smith and Springborn studies covers the critical windows for exposure of pre-school and school children. As such, the PHG NOAEL should be used to develop a child-specific RD for use in school-site risk assessment.

OEHHA has considered the appropriateness of using an oral absorption factor for calculating the chRD for nickel. OEHHA noted that human absorption of nickel depends on the dietary matrix (OEHHA 2001). Absorption is significantly higher when water is used as the administration vehicle, compared to when food is used. Alexander et al. (1974) estimated a 40 percent absorption for healthy children on a balanced diet that consisted of milk, cereal, and other food. NcNeely et al. (1972), on the other hand, estimated a 1.6 percent absorption for adults on a regular diet (water and food). These data suggest the appropriateness of applying a child protective factor of three to account for the absorption difference between children and adults.

Because a PHG can be based on a cancer or non-cancer endpoint, OEHHA applied a factor of 10 to account for database deficiencies for carcinogenic effect via oral route in deriving a PHG safe dose for nickel. Since a non-cancer chRD by definition addresses the non-cancer endpoint only, OEHHA in this situation has not applied that database deficiency factor in calculating the chRD for nickel.

Calculation of the chRD for nickel is based on the following equation:

$$\text{chRD} = \frac{\text{NOAEL}}{\text{UF x CP}} = \frac{1.1\ \text{mg/kg-day}}{100 \times 3} = 3.7\ \mu\text{g/kg-day}$$

Where,

NOAEL =	No-observed-adverse-effect-level from Smith et al., 1993; Springborn Laboratories, 2000a, b.
UF =	Uncertainty factor of 100 (10 for inter-species extrapolation, 10 for human variability).
CP =	Child protective factor of 3 to account for the GI absorption difference between children and adults.

Accordingly, OEHHA is proposing a non-cancer chRD of 3.7 μg/kg-day for nickel to be used in school-site risk assessment.

3. Conclusion

This report summarizes OEHHA's evaluation of cadmium, chlordane, heptachlor (and its metabolite heptachlor epoxide), methoxychlor, and nickel. Based on the evaluation, OEHHA proposes to establish a chRD for each of these chemicals pursuant to the second part of Health and Safety Code Section 901(g). They are listed in Table 3.1 along with other pertinent numerical health criteria.

Table 3.1 Numerical Non-cancer Health Criteria

	OEHHA's Proposed chRD (mg/kg-day)	OEHHA's PHG Safe Dose (mg/kg-day)	U.S. EPA's RfD (mg/kg-day)
Cadmium	1×10^{-5}	1×10^{-5}	5×10^{-4}
Chlordane	3.3×10^{-5}	1×10^{-5}	5×10^{-4}
Heptachlor	3×10^{-5}	1×10^{-4}	5×10^{-4}
Heptachlor epoxide	1.3×10^{-5}	1.3×10^{-5}	1.3×10^{-5}
Methoxychlor	2×10^{-5}	5×10^{-3}	5×10^{-3}
Nickel	3.7×10^{-3}	1.1×10^{-3}	2×10^{-2}

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APPENDIX A

Keywords Used in Literature Search

gestation*
infant
neonatal
neonate*
newborn
perinate
perinatal
perinatally
lactation
puberty
adolescent
adolescence
kids
young
youth
children
child
juvenile
school
pediatric
prepubertal
peripubertal
age
sacrificed
lactation
pup
pups
postnatal*
preweanling
weanling*
early postnatal exposure
offspring
immature
childhood
developmental
growth
developing
development
rotarod
utero
early postnatal exposure
synaptogenesis
cell physiology
myelination
myelin sheath
apoptosis
locomotor skill
motor activity
learning
Psychological
Phenomena and
Processes
memory
pseudoglandular
canalicular
saccular
morphogenesis
Respiratory Tract
Diseases
Splenic diseases
spleen
hematopoeisis
extramedullary
thymus gland
autoimmunity
endocrine glands
brain
gonads
ovary
testis
urogenital system
kidney
ureters
bladder
urethra
ovaries
uterus
fallopian tubes
vagina
clitoris
testes
seminal vesicles
prostate seminal ducts
penis
breast/gd
mammae
udder
sperm count
sperm motility
sex maturation
vaginal opening
preputial separation
litter size
Estrogens
androgens
Leydig Cell Tumor
Leydig Cells
Sertoli Leydig Cell
Tumor
Sertoli Cell Tumor
Sertoli Cells
maze learning
sex hormones
steroid
receptors
GABA
body weight
cincinnati maze
navigation times
escape reaction
Startle reaction
startle
spatial behavior
crowding
personal space
territoriality
mating behavior

sex behavior
motor activity
chloride channels
gaba receptors
auditory startle
Neuropsychological Tests
Reaction Time
Psychomotor Performance
Battery
Physiology
Nervous System
Psychological Phenomena and Processes
Behavior and Behavior Mechanisms
Psychological Tests
Behavioral Disciplines and Activities
Ovarian Function Tests
Pain Measurement
placental Function Tests
Pulmonary Ventilation
Respiratory Function Tests
Speech Articulation Tests
Speech Discrimination Tests
Thyroid Function Tests
Pancreatic Function Tests
Ethology
hearing tests
vision
visual perception
ethological
photic stimulation
uterotrophic
Immune system
immunity
immunotox*
Nervous system
nervous system diseases
Neurologic Manifestations
neurotox*
Respiration system
Respiratory Tract Diseases
respirat*
lung
lungs
nasal
airway
Neurosecretory Systems
neuroendocrin*
Psychomotor Agitation
Neurobehavioral Manifestations
Psychomotor Performance
Psychophysiology
behavior
neurobehav*
reproduction